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## Bone and Skeletal Muscle: Key Players in Mechanotransduction and Potential Overlapping Mechanisms

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### Abstract

The development and maintenance of skeletal muscle and bone mass is critical for movement, health and issues associated with the quality of life. Skeletal muscle and bone mass are regulated by a variety of factors that include changes in mechanical loading. Moreover, bone mass is, in large part, regulated by muscle-derived mechanical forces and thus by changes in muscle mass/strength. A thorough understanding of the cellular mechanism(s) responsible for mechanotransduction in bone and skeletal muscle is essential for the development of effective exercise and pharmaceutical strategies aimed at increasing, and/or preventing the loss of, mass in these tissues. Thus, in this review we will attempt to summarize the current evidence for the major molecular mechanisms involved in mechanotransduction in skeletal muscle and bone. By examining the differences and similarities in mechanotransduction between these two tissues, it is hoped that this review will stimulate new insights and ideas for future research and promote collaboration between bone and muscle biologists.

### Keywords

hypertrophy; mTORC1; growth; bone mineral density; mechanical loading

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## INTRODUCTION

Skeletal muscle and bone play fundamental roles in human physiology, enabling locomotion and movement, enhancing blood flow to organs, and providing protection to vital organs, among others. Beyond the mechanical roles of these two organ systems, both are also major regulators of whole body metabolism. For instance, skeletal muscle serves as a storage site/consumer of amino acids and glucose, and secretes various myokines that affect metabolism in other tissues [1–3]. Bone serves as an ion bank for maintaining serum levels of physiologically crucial elements such as  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , and also secretes active endocrine products [4–6]. In light of the far-reaching roles of these tissues in general health, it is imperative that the field comes to a better understanding of the conditions that concomitantly affect muscle and bone health, most notably reduced bone and/or skeletal muscle mass (either during pre/post-natal development or in adults). These conditions have the potential to increase the risk of injury and metabolic disease, reduce physical mobility, and ultimately affect the quality and duration of life.

Skeletal muscle mass and bone mass are regulated by a range of factors that include genetics, nutrition, hormones and growth factors and, in particular, mechanical stimuli [7, 8]. It is well known that an increase in mechanical loading of skeletal muscle results in an increase in skeletal muscle mass (i.e., muscle hypertrophy), while a decrease in mechanical loading leads to a reduction of skeletal muscle mass (i.e., muscle atrophy) (for reviews see [9–11]). The mechanical loading experienced by skeletal muscle typically comprises the internal longitudinal and lateral forces, of varying magnitudes and velocities, which are generated by active muscle contractions (e.g., shortening, lengthening, or isometric contractions) or by passive stretch. Changes in mechanical loading are also known to play a major role in the regulation of bone mass and strength; increased mechanical loading at critical stages of growth and development result in increased bone mineral accrual, bone mass and strength, while reduced mechanical loading results in the loss of bone mass and strength (for reviews see [12–14]). Importantly, evidence suggests that the development and maintenance of bone mass is, in large part, dependent on skeletal muscle-derived mechanical loading [15, 16].

Skeletal muscles contribute to the mechanical loading of bone in various ways that include the tensile forces developed by contracting muscles at their site of insertion, the compressive forces between bones developed by muscles contracting across joints, and bending forces experienced by long bones as muscles generate force for lifting distally held objects [15]. In support of a critical role for skeletal muscle-induced mechanical stimuli in the regulation of bone mass, studies have shown that a lack of muscle function in utero results in impaired fetal bone and joint development [17–21]. Furthermore, during post-natal skeletal growth (2–20 yr), there is a very strong positive correlation between muscle mass and bone mass, with gains in muscle mass preceding those in bone mass [22]. In fact, the associations between muscle/strength and bone mass in children are strong enough that clinical techniques for disease diagnosis can be founded upon them [23, 24]. For instance, deficiencies in the amount of bone per unit muscle strength versus deficiencies in both factors allow for classification of diagnoses into primary (true or intrinsic) and secondary

(physiologic) bone disorders. Combined, these data strongly suggest that greater forces, produced by larger muscles, may play a direct role in stimulating bone growth. Conversely, states of reduced muscle mass and function, such as various neuromuscular diseases (e.g. cerebral palsy and Duchenne muscular dystrophy [25, 26]), spinal cord injury [27] and space flight [28], are all associated with a loss of bone mass. It is also interesting to note, and perhaps telling with respect to the influence of muscle forces on bone mass, that in many disease states, bone mass is typically not over adapted for muscle mass [29].

Despite the fundamental dependence on mechanical stimuli for the development and/or maintenance of bone and skeletal muscle mass, the exact mechanism(s) by which changes in mechanical loading are transduced into anabolic or catabolic signaling events (i.e, mechanotransduction) in these tissues remains to be fully determined. A thorough understanding of the cellular mechanism(s) responsible for mechanotransduction in bone and skeletal muscle is essential for the development of effective exercise and pharmaceutical strategies aimed at increasing, and/or preventing the loss of, mass in these critical tissues. Furthermore, it is clear that a sizeable and powerful complement of skeletal muscle and a robust and rigid skeleton are desirable outcomes for optimal connective tissue health. Therefore, it is worthwhile to understand the molecular underpinnings of mechanotransduction in both tissues and to determine whether both can be optimized in tandem [30]. Hence, the purpose of this review is to summarize our understanding of the key mechano-sensitive signaling events that are thought play a role in the regulation of bone and skeletal muscle mass. Importantly, we will also highlight important gaps in the body of knowledge in the hope that this will stimulate further research, and potentially collaboration between bone and muscle biologists.

## **MECHANOTRANSDUCTION AND THE REGULATION OF SKELETAL MUSCLE MASS**

Skeletal muscle mass is ultimately determined by the net difference in the rates of protein degradation and protein synthesis [31]. For example, a net increase in protein synthesis and/or a net decrease in protein degradation leads to muscle hypertrophy, while a net decrease in protein synthesis and/or increase in protein degradation results in muscle atrophy. Importantly, mechanical load-induced changes in muscle mass are associated with changes in protein synthesis, with increased mechanical load-induced muscle hypertrophy being associated with an increase in protein synthesis [10]. Despite the major role that protein synthesis plays in the mechanical regulation of skeletal muscle mass, the molecular mechanism(s) through which changes in mechanical loading regulate protein synthesis, and thus muscle mass, remain to be fully determined. Nevertheless, progress is being made and a significant body of evidence now indicates that the protein kinase called the mechanistic/mammalian target of rapamycin (mTOR) plays a central role in the pathway through which mechanical stimuli regulate protein synthesis and muscle mass [9, 11]. As such, the remainder of this section will focus on the role of mTOR in mechanically-induced increases in protein synthesis and muscle mass, and on several putative mechanically-sensitive factors that are proposed to play a role in the activation of mTOR signaling. It is important to note, however, that other signaling mechanisms also play important roles in the regulation of

protein synthesis and skeletal muscle mass during different stages of growth and development and under various conditions that may include increased mechanical loading [for recent reviews on these topics, see [32–41]. Furthermore, although somewhat controversial, there is also evidence that proliferation, differentiation and fusion of satellite cells (SC) may play a role in the mechanical regulation of skeletal muscle mass; however, the exact mechanism(s) responsible for the mechanically-induced activation of SCs remains to be determined [10, 42–45].

### **The Central Role of mTOR/mTORC1 in Mechanically-Induced Skeletal Muscle Growth**

mTOR is a conserved serine/threonine kinase that is found in at least two multi-protein complexes: mTOR complex 1 (mTORC1), whose signaling is partly inhibited by the drug rapamycin, and mTORC2, which is largely rapamycin-resistant [46]. mTORC1 is a major regulator of cell growth, in part, by regulating mRNA translation, and thus protein synthesis, with the potential to regulate both translational efficiency (i.e., the rate of mRNA translation) and translational capacity (i.e., the number of ribosomes) (for a review, see [47]). Importantly, in skeletal muscle, mTORC1 signaling has been shown to be activated by a wide-range of different types of mechanical stimuli, and this effect can be observed in cell culture, and in whole muscle *ex vivo* and *in vivo* model systems [9]. Moreover, it has been demonstrated that the *in vivo* activation of mTORC1 signaling is sufficient to stimulate an increase in protein synthesis and induce muscle fiber hypertrophy [48, 49], and that the kinase activity of mTOR is necessary for mechanical load-induced muscle hypertrophy [50, 51]. However, despite the robust evidence for mTORC1's central role in mechanically-induced skeletal muscle growth, the identification of the upstream mechanism(s) that are responsible for the mechanical activation of mTORC1 remains a significant challenge. Below we will briefly describe the evidence for, and against, several putative mechanically-induced activators of mTORC1 signaling in skeletal muscle

### **Potential Candidates for the Mechanically-Induced Activation of mTORC1**

**Insulin-Like Growth Factor 1**—One of the earliest molecules that was proposed to be involved in the mechanical activation of mTORC1 signaling is the insulin-like growth factor 1 (IGF-1). For example, early studies showed that IGF-1 expression was increased by mechanical loading, including a splice variant known as the mechano growth factor (MGF) which was proposed to act in an autocrine manner [52–55]. IGF-1 overexpression was also shown to be sufficient to activate PI3K/Akt/mTORC1 signaling, increase protein synthesis and induce muscle hypertrophy [56–65]. While there is some limited evidence that IGF-1 may play a role in the increase in protein synthesis several hours after resistance exercise in diabetic muscle [66], more recently, a significant body of evidence has accumulated which indicates that mechanical stimuli activate mTORC1 signaling, at least initially, via an IGF-1/PI3K/Akt-independent mechanism, and that IGF-1/PI3K/Akt signaling is not necessary for mechanically-induced skeletal muscle growth (for a more detailed review of this topic see [9]). Thus, while IGF-1 plays an important role in the development and/or maintenance of skeletal muscle mass [67], current evidence suggests that IGF-1 does not play a significant role in the acute mechanical activation of mTORC1 and that IGF-1 is not necessary for mechanically-induced skeletal muscle hypertrophy. Further work is required to definitively

determine the role, if any, of IGF-1 in the acute and more prolonged activation of mTORC1 and protein synthesis, and in muscle hypertrophy, in response to mechanical stimuli.

**Extracellular Signal-Regulated Kinase 1 and 2**—Another potential mechanically-activated upstream regulator of mTORC1 and skeletal muscle mass is the extracellular signal-regulated kinases 1 and 2 (ERK1/2). These members of the mitogen-activated protein kinase (MAPK) family form part of the Ras/Raf/MEK/ERK1/2 signaling pathway and are activated by various types of mechanical stimuli in cultured muscle cells and in rodent and human skeletal muscle *in vivo* [68–74]. ERK1/2-mediated signaling has the potential to positively regulate protein synthesis, in part, by the activation of mTORC1 signaling via p90RSK-mediated phosphorylation of TSC2 and Raptor [75–80]. While these studies suggest that ERK1/2 signaling could play a role in the mechanical activation of mTORC1 signaling, protein synthesis, and muscle hypertrophy, it was recently shown that ERK1/2 inhibition does not prevent stretch-induced hypertrophy in cultured myotubes [71]. Furthermore, while inhibition of ERK1/2 reduced basal mTORC1 signaling, it did not inhibit the stretch-induced increase in mTORC1 signaling or protein synthesis in isolated mouse muscles [74]. Thus, based on a limited number of studies, it appears that ERK1/2 signaling is not necessary for the mechanically-induced activation of mTORC1 signaling, protein synthesis, or muscle hypertrophy. Further research in rodents and humans, using different experimental models, is needed to further clarify these findings.

**Phosphatidic Acid**—In the absence of convincing evidence for either IGF-1 or ERK1/2 in the mechanical activation of mTORC1, protein synthesis or skeletal muscle growth, recent studies have begun to focus on the glycerophospholipid second messenger, phosphatidic acid (PA). PA is a direct activator mTORC1 signaling, most likely via its ability to bind to the FKBP12-rapamycin binding (FRB) domain of mTOR [74, 81, 82]. Studies have shown that the stimulation of cells with exogenous PA, or the overexpression of PA-generating enzymes, such as phospholipase D1 (PLD1), lysophosphatidic acid acyltransferase  $\theta$  (LPAAT $\theta$ ) and diacylglycerol kinase  $\zeta$  (DGK $\zeta$ ), activates mTORC1 signaling [74, 83–86]. Furthermore, the overexpression of PLD1 and DGK $\zeta$  has been shown to be sufficient to induce skeletal muscle fiber hypertrophy in mice [87, 88]. Importantly, mechanical stimulation of skeletal muscle (i.e. passive stretch and eccentric contractions) induces an increase in muscle [PA] [74, 85, 88], suggesting that PA could indeed play a role in the mechanical activation of mTORC1. Initial studies investigating this hypothesis focused on the potential role of PLD as the source for the mechanically-induced increase in PA [85, 89]; however, more recent studies using a specific small-molecule PLD inhibitor found that PLD activity was not necessary for passive stretch-induced increases in PA [88]. Instead, it was found that passive stretch induced an increased in membrane associated DGK activity. Furthermore, in DGK $\zeta$  knockout mice, it was shown that the stretch-induced increase in PA is almost completely abolished and that the activation of mTORC1 signaling is markedly impaired [74]. Thus, current evidence strongly suggests that DGK $\zeta$  is predominantly responsible for the stretch-induced increase in PA and is a major contributor to the mechanical activation of mTORC1 signaling. Further research is still required, however, to confirm the role of DGK $\zeta$  in other models of mechanical stimulation (e.g.

eccentric contractions and synergist ablation-induced mechanical overload) and to determine whether DGK $\zeta$ -derived PA is sufficient to increase protein synthesis.

**TSC2 Translocation and Rheb GTP-Loading Status**—TSC2 and Rheb (Ras homologue enriched in the brain) form part of the canonical insulin/IGF-1 signaling pathway [90]. Rheb, located immediately upstream of mTOR, is a GTP-binding protein that possesses GTPase activity and its GTP/GDP-binding status is regulated by the GTPase activating protein (GAP) activity of TSC2 (a.k.a. Tuberin) [91]. Rheb interacts with mTOR's catalytic domain, and when in its GTP-bound state, Rheb can directly activate mTOR kinase activity [92, 93]. It is currently thought that, under basal conditions, the GAP domain of TSC2 stimulates Rheb's intrinsic GTPase activity which converts active GTP-Rheb into inactive GDP-Rheb, and thus leads to a repression of mTORC1 signaling [94–96]. Conversely, when stimulated by factors such as insulin, TSC2 GAP activity is proposed to be inhibited in a phosphorylation-dependent manner, leading to an increase in GTP-Rheb and the activation of mTORC1 signaling [90]. In skeletal muscle, the overexpression of Rheb is sufficient to activate mTORC1 signaling, induce an increase in protein synthesis, and stimulate muscle fiber hypertrophy [48, 49]. Despite this, there is currently very little known about the potential role of TSC2 and Rheb in the mechanical activation of mTORC1 signaling in skeletal muscle. Recently, however, it was shown that an acute bout of eccentric contractions almost completely abolished the association of TSC2 with late endosomal/lysosomal (LEL) structures [97]. As LEL structures are also the location of a population of mTOR and Rheb (for review see [98]), this finding suggests that the mechanically-induced translocation of TSC2 away from the LEL could lead to an increase in the amount of GTP-loaded Rheb at the LEL and subsequently promote the activation of mTORC1 signaling. While these findings appear promising, it remains to be determined whether: 1) the translocation of TSC2 away from the LEL plays an important role in the mechanical activation of mTORC1 signaling; 2) whether TSC2 translocation is mediated through a phosphorylation-mediated event and, if so, what kinase(s) are responsible for the changes in TSC2 phosphorylation; and 3) whether Rheb is necessary for the mechanical activation of mTORC1 signaling, protein synthesis and hypertrophy.

**Increased Intracellular Calcium**—Skeletal muscle contractions are initiated by an action potential-induced release of calcium ( $\text{Ca}^{2+}$ ) from the sarcoplasmic reticulum (SR) and the subsequent  $\text{Ca}^{2+}$ -induced activation of the contractile apparatus [99]. Furthermore, passive stretch of myoblasts and skeletal muscles can also result in an increase in intracellular  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ), most likely via the activation of stretch sensitive ion channels [100, 101]. Importantly, studies in non-muscle cells have shown that mTORC1 signaling may be regulated by changes in  $[\text{Ca}^{2+}]_i$ , with an increase in  $[\text{Ca}^{2+}]_i$  activating mTORC1 signaling and a decrease in  $[\text{Ca}^{2+}]_i$  leading to an inhibition of mTORC1 signaling [102, 103]. Increased  $[\text{Ca}^{2+}]_i$  has also been shown to increase *ex vivo* rates of protein synthesis in resting skeletal muscle [104]. Thus, contraction- or stretch-induced increases in  $[\text{Ca}^{2+}]_i$  would appear to be an ideal candidate for the mechanical activation of mTORC1 and protein synthesis in skeletal muscle. However, despite the potential role for  $[\text{Ca}^{2+}]_i$ , few studies have attempted to directly examine this hypothesis. Firstly, it was reported that the  $\text{Ca}^{2+}$  chelator, BAPTA-AM, did not inhibit *ex vivo* stretch-induced mTORC1 signaling in isolated



skeletal muscles [89]. In contrast, the *in vivo* inhibition of  $\text{Ca}^{2+}$  (and  $\text{Na}^{+}$ ) permeable stretch-activated ion channels with gadolinium ( $\text{Gd}^{3+}$ ) was shown to inhibit the eccentric contraction-induced increase in  $[\text{Ca}^{2+}]_i$  and  $\text{Gd}^{3+}$  attenuated eccentric contraction-induced mTORC1 signaling [105, 106]. More recently, it was shown that the *in vivo* mechanical overload-induced activation of mTORC1 signaling and muscle hypertrophy was stimulated by an increase in  $[\text{Ca}^{2+}]_i$  via the activation of SR transient receptor potential cation channel subfamily V (TrpV1) channels [107, 108]. Mechanistically, a contraction/stretch-induced increase in  $[\text{Ca}^{2+}]_i$  could potentially stimulate mTORC1 signaling and protein synthesis by the activation of the  $\text{Ca}^{2+}$ /calmodulin-dependent protein kinase  $\alpha$  (CaMKK $\alpha$ ) which has been shown to up regulated by chronic mechanical overload [109, 110]. Furthermore, constitutively active CaMKK $\alpha$  was found to be sufficient to activate mTORC1 signaling, increase protein synthesis in a rapamycin-sensitive manner and induce muscle hypertrophy; however, paradoxically CaMKK $\alpha$  was also found to not be necessary for overload-induced muscle growth [110]. Thus, overall, while increases in  $[\text{Ca}^{2+}]_i$  are an attractive candidate for the mechanical activation of mTORC1, further work is required to reconcile some of the conflicting results obtained to date, and to identify the molecular mechanism(s) through which changes in  $[\text{Ca}^{2+}]_i$  could regulate mTORC1 signaling.

**Reactive Nitrogen Species**—Studies in non-muscle cells have shown that mTORC1 signaling can be regulated positively and negatively by changes in the cellular redox state [111]. It is well known that the acute mechanical stimulation of skeletal muscle results in changes in the cellular redox state via the production of reactive oxygen species [ROS; e.g., superoxide ( $\text{O}_2^-$ ) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ )] and reactive nitrogen species [RNS; e.g., nitric oxide (NO) and peroxynitrite ( $\text{ONOO}^-$ )] [112]. Therefore, changes in the production of ROS and/or RNS are potential candidates for the mechanical activation of mTORC1 signaling and skeletal muscle hypertrophy. In support of this possibility, it has been shown that the nitric oxide synthase (NOS) inhibitor, L-NAME, can inhibit stretch-induced hypertrophy of cultured myotubes and synergist ablation-induced muscle hypertrophy in rats [113–115]. Furthermore, muscles from neuronal NOS (nNOS)-null mice fail to undergo synergist ablation-induced muscle hypertrophy [107]. The exact mechanism behind the apparent role of NO in mechanically-induced hypertrophy remains to be determined, but recent evidence suggests that it may, in part, be due to increases in  $[\text{Ca}^{2+}]_i$  [107]. Specifically, it was concluded that the synergist ablation-induced increase in  $[\text{Ca}^{2+}]_i$  and mTORC1 signaling is stimulated by the formation of  $\text{ONOO}^-$  that is produced from nNOS-derived NO and NADPH oxidase 4 (Nox4)-derived  $\text{O}_2^-$  [107, 108]. Although very attractive, significantly more work using different experimental models will be required to fully define the role of RNS in the mechanical activation of mTORC1 and skeletal muscle growth. Interestingly, nNOS-derived NO may also play a role in muscle atrophy induced by a reduction in mechanical loading [116]. For example, it was recently shown that hindlimb suspension-induced muscle atrophy was associated with the dislocation of nNOS from the membrane associated dystrophin glycoprotein complex to the cytoplasm and with increased NO production. Furthermore, the muscle atrophy was inhibited in nNOS-null mice and in mice treated with the nNOS-specific inhibitor, 7-nitroindazole [116]. Thus, RNS appear play a role in the regulation of muscle mass in response to both increased and decreased

mechanical loading. Further work is required to determine whether there is any link between muscle atrophy, NO and mTORC1 signaling.

**Amino Acids**—Amino acids are well known regulators of mTORC1 signaling, with amino acid depletion reducing mTORC1 signaling and increased amino acid availability leading to increased mTORC1 signaling [117]. It was recently demonstrated in non-muscle cells that amino acids regulate the association of mTOR with LEL structures via a mechanism that is dependent on the Rag family of GTPases [118–120]. Specifically, amino acid stimulation increases the association of mTOR with LEL structures by regulating the activity/GTP-loading state of the Rag GTPases [121]. Based on these data, it has been suggested that amino acid-induced changes in mTORC1 signaling are primarily regulated by spatially controlling the ability of mTOR to interact with the LEL-associated activator, Rheb. In skeletal muscle, amino acids (especially the branched-chain amino acid, leucine) are sufficient to activate mTORC1 signaling and increase protein synthesis via a rapamycin-sensitive mechanism [122, 123]. Therefore, it is plausible that an increase in the uptake of amino acids could play a role in the mechanical activation of mTORC1 signaling. To date, however, there is currently no direct evidence to support this hypothesis. Nevertheless, recent studies have shown that acute resistance exercise increases both mTORC1 signaling and the content of leucine in rat muscle [124], and increases the post-exercise activity of the class III PI3K, Vps34, which has been implicated in the amino acid-induced activation of mTORC1 signaling [125–128]. On the contrary, a recent study has reported a decrease in endogenous muscle leucine content after acute resistance exercise despite an increase in mTORC1 signaling in middle aged humans [129]. Thus, while some indirect evidence suggests that amino acids could play a role in the mechanical activation of mTORC1 signaling, significantly more research is required to gain further insights into this possibility.

In summary, a significant body of evidence has established that mTORC1 plays a central role in the mechanical regulation of protein synthesis and skeletal muscle mass. Currently, there are several potential candidate molecules that have been proposed to play a role in the pathway through which mechanical stimuli activate mTORC1 signaling. While most of these molecules are likely to play important roles in the regulation of mTORC1 signaling, protein synthesis and muscle mass during different stages of growth and development, current evidence suggests that DGK $\zeta$ -derived PA plays a major role in the acute mechanical activation of mTORC1. This role, however, is not necessarily exclusive. Indeed, future research may identify that a complex range of factors contribute to the full mechanical activation of mTORC1 signaling. For example, the mechanically-induced dissociation of TSC2 from the LEL and the subsequent activation of Rheb, an increase in intracellular amino acids, and/or RNS-induced SR Ca<sup>2+</sup> release may all combine with DGK $\zeta$ -derived PA to promote the full activation of mTORC1 signaling. Ultimately, the relative contribution of each activating factor is likely to be specific to the type, intensity and duration of the mechanical stimulus, and to the time point examined following the initiation of the stimulus. This potential complexity highlights how limited our current understanding is, and underscores the need for further research into the process of mechanotransduction on regulation of mTORC1, protein synthesis and skeletal muscle mass. Furthermore, significantly more research is required to identify the upstream mechano-sensitive



element(s)/receptor(s) that ultimately stimulate the array of putative mTORC1 activators reviewed above.

## MECHANOTRANSDUCTION AND THE REGULATION OF BONE MASS

### Mechanoreception and tissue adaptation in bone: a different mechanism than muscle, involving division of labor among cell types

While skeletal muscle mass is ultimately determined by the net difference in the rates of protein degradation and protein synthesis within the cell, the regulation of bone mass represents a more complicated phenomenon because bone tissue is incapable of interstitial growth [130]. That is, the cross sectional size of a given muscle can change via hypertrophy or atrophy of preexisting myofibers, whereas cellular hypertrophy/atrophy of bone cells is not a mechanism that directly augments bone mass. In bone, changes in the mineralized matrix must be achieved by specialized cells (osteoblasts and osteoclasts) that are at the beck and call of the embedded master-regulatory cell type - the osteocyte [131]. Because changes in bone mass are enacted by the elaboration or removal of a complicated matrix, changes in bone mass occur much more slowly than the cell-trophic changes that occur in muscle tissue. Moreover, the physical environment of the osteocyte is completely different than that of bone cells localized on the bone surface. Osteocytes are entombed in a small, form-fitting cavity within the bone matrix (the lacuna). As the osteocyte cell processes emerge and course away from the cell body and lacuna, they travel in canaliculi (nano-canals) within the bone matrix [132]. Decades ago, it was postulated that the osteocyte was the best candidate for a sensor cell type, for several reasons [133]. First, the osteocytes are regularly distributed throughout cortical and trabecular bone, even in areas of mineralized matrix devoid of vasculature. Consequently, the network of osteocytes provides a widespread load-monitoring “net” that infiltrates every cubic millimeter of bone tissue. Second, osteocytes are connected to one another, and to bone surface cells, through long cellular processes that course through the bone and project to the bone surfaces. Osteocytes have a large number of these cell processes (~50/cell) emanating from the cell body and coursing in all directions [134]. The cell processes join similar cell processes from neighboring osteocytes and transmit information inter-cellularly *via* gap junctions, which facilitate rapid cell-cell communication [135]. Third, it is clear that osteocytes are not effector cells, as they are entombed in a bony matrix and therefore are incapable of adding or removing very much matrix, and can only remove it in the small area surrounding their lacunae [136]. This very localized activity of osteocytes, while potentially meaningful for regulating serum calcium levels, has little to no effect on bone size, shape, and structural properties. Because their role as an effector cell is precluded, they have been thought of historically (somewhat by default) as a sensor cell [137]. Beyond teleological arguments, experiments have supported the role of the osteocyte as the primary mechanosensory cell type in bone. Interestingly, a recent gene expression profile conducted on purified, flow-sorted populations of osteocytes extracted from living mouse bone, revealed a surprising number of highly expressed genes that are traditionally thought of as “muscle genes” [138]. While that experiment was not conducted under mechanically altered conditions, it is also worthwhile to consider that osteocytes might be more “muscle-like” than previously

thought, and that some of the mechanisms of strain sensation might overlap between these two cell types.

### Ion Channels

Many mechanosensitive tissues in the body are regulated by ion channels. Ion channels are pore-forming proteins that traverse the plasma membrane and allow ions to flow into and out of a cell based on electrochemical gradient. Their activity (opening and closing) can be controlled by different mechanisms; among them are changes in voltage across the membrane, biochemical ligands, or physical stimuli such as mechanical perturbation of the membrane. It is well documented experimentally that ion channels play a crucial role in the process of mechanotransduction in bone [139, 140]. For example,  $Gd^{3+}$  is a potent blocking agent for mechanosensitive ion channels, and pretreatment of bone cells with  $Gd^{3+}$  prior to mechanical loading stimulation results in a loss of downstream mechanotransduction marker expression [141, 142]. However,  $Gd^{3+}$  is a fairly nonspecific inhibitor (e.g., some L-type voltage sensitive calcium channel inhibition has been reported), so it is difficult to be certain that the inhibitory effect of  $Gd^{3+}$  on mechanotransduction involves a mechanosensitive ion channel. More recently, the transient receptor potential (Trp) family of channels has been investigated regarding their role in bone cell mechanotransduction [143, 144]. Specifically, TrpV4, a receptor known to be sensitive to mechanical perturbation (most notably, cell swelling) and osmolarity in other tissues [145], was recently shown to modulate the response to mechanical disuse in mice. TrpV4 knockout mice subjected to tail suspension for 2 wk failed to lose bone and did not exhibit reduced bone formation rates, as was observed in tail suspended wild type mice [146]. The Trpv4 channel has been shown to be sensitive to shear stress in other cell types, thus its role in bone as a mechanosensor is promising. Another Trp channel that has received significant attention in bone cell mechanotransduction is TrpP1, also known as polycystin-1 (encoded by the *Pkd1* gene). Several years ago, it was convincingly demonstrated that that polycystin-1 and polycystin-2 regulate mechanotransduction in kidney epithelial cells [147]. Polycystin-1 and -2 reside largely on the primary cilium—a nonmotile ~250 nm thick cytoskeletal stalk that extends into the tubular lumen [148]. Deflection of the cilium from fluid movement in the renal tubules activates polycystin-1, which causes the polycystin-2 channel to open, kicking off a  $Ca^{2+}$  cascade that has multiple downstream effects [149]. For example, in osteocyte-specific polycystin-1 knockout mice, *in vivo* ulnar loading exhibited a ~70% reduction in load-induced apposition rates compared to control mice, indicating that osteocytic polycystin-1 is an important protein in the anabolic response to skeletal loading [150]. Similar effects have been found *in vitro*, where cilium disruption via chemical treatment or gene silencing inhibits the response to matrix strain [151]. In light of the importance of fluid movement in the canaliculo-lacunar network, the primary cilium, and its complement of associated proteins, is an attractive candidate for mechanosensing in the osteocytes. But it is difficult to envisage how the cilium would physically fit in the extracellular space because the distance between the canalicular wall and the osteocyte cell process/body is typically 50–80 nm and could be much smaller if measured on sections processed with newly refined fixation techniques that reduce cell shrinkage [152]. In summary, there is evidence suggesting that the primary cilium itself, or perhaps proteins normally associated with the cilium, mediate

mechanotransduction in bone; but greater experimentation in this area will be required to more fully understand the precise role of cilia in the mechanical signaling process.

### G-protein coupled receptors

G-protein coupled receptors (GPCRs) represent the largest family of cell surface receptors, and are activated by a variety of ligands including neurotransmitters, hormones, small peptides, local cytokines, amino acids, and fatty acids, among others (reviewed in [153]). More than a decade ago, it was demonstrated that fluid flow activates G-proteins in osteoblasts, and that pharmacologically preventing G-protein activation prevents the normal response to fluid shear [154]. It is interesting to note that fluid shear stress leads to a conformational change in two GPCRs - the parathyroid hormone 1 receptor (PTH1R) and the B2 bradykinin receptor, in MC3T3 osteoblastic cells and BAEC endothelial cells [155, 156]. This result was detected using GPCR conformation-sensitive fluorescence resonance energy transfer (FRET), which revealed conformational changes in these GPCRs occurring within milliseconds of shear exposure. The changes were independent of the presence of either receptor's ligand. It was also reported that the responsiveness of the energy transfer signal could be modulated by membrane fluidity (e.g. modulation of membrane stiffness), indicating that these GPCRs might be direct sensors of mechanical perturbation of the membrane. While mechanotransduction may or may not involve either of these two particular receptors, the data make the larger point that other GPCRs, more crucial to the mechanotransduction response, might undergo similar conformational changes when the cell is mechanically stimulated. The premise that a ligand is not necessary for shear to activate intracellular G-proteins has been taken one step further, and it might be true that even the receptor itself is not necessary for shear-induced G-protein activation. For instance, the same group showed that when purified G-proteins are reconstituted into otherwise empty phospholipid vesicles, they could be activated (GDP hydrolysis) almost immediately upon fluid shear [157]. This flow-induced activation was independent of a GPCR presence, but rather, was modulated by membrane stiffness. Although, bone cells possess the G-protein machinery necessary to activate this pathway, verification of this mechanoreception mechanism in bone cells *per se* will require similar experiments in bone-specific models, which have not been done to date.

The search for the “mechanoreceptor” in bone is ongoing, with progress being made on several fronts. The molecule or mechanism that is at the forefront of mechanoreception—the protein that converts a physical signal into a biochemical signal—remains elusive. It is also possible that no single mechanism is responsible for initiating the entire event; numerous signaling systems in biology exhibit redundancy. While the mechanisms of signal reception are still being worked out, much greater progress has been made in the field of mechanically stimulated second messengers that are required for mechanically-induced changes in bone mass. Those mechanisms are described next.

### Mechanically stimulated second messengers in bone

Once the mechanical signal is received by the local bone cell population and translated into an initial biological signal, a series of secondary biochemical signaling events must occur to propagate the signal within the cell and to other sensor/effector cells. Efforts to understand

the signaling pathways involved in mechanical signal propagation have uncovered a multitude of changes in the mechanically stimulated osteocyte/osteoblast, including gene expression changes, protein and lipid modifications (e.g., phosphorylation events), protein degradation, intracellular translocation events, release of secreted factors, and alterations in cell shape and size, among others. The challenge presented by these observations is to determine which among them are critical for mechanotransduction to occur, and which are simply auxiliary events that have few functional consequences for the mechanotransduction process.

**Prostaglandins**—One of the earliest pathways identified to be involved in bone cell mechanotransduction is the cyclooxygenase (Cox)/prostaglandin (PG) pathway. PGs are potent and short lived (sec/min) arachidonic acid (AA)-derived signaling molecules that act in an autocrine/paracrine manner [158]. In a multistep process, intracellular cyclooxygenase (Cox) enzymes convert AA to PGG<sub>2</sub>, and then to PGH<sub>2</sub>, after which various PG synthases generate specific PGs including PGE<sub>2</sub> [158]. PGs are secreted from the cell in response to a number of stimuli where they can then bind to specific GPCRs. Vigorous jumping exercises in humans induce an immediate release of PGE<sub>2</sub> from lower limb (loaded) bone tissue [159]. In rodents, mechanical loading up-regulates the mRNA and protein levels of Cox2 (the inducible isoform of Cox), whereas the constitutive isoform (Cox1) remains unchanged [160, 161]. The importance of PGE<sub>2</sub> signaling has been demonstrated *in vivo* by depleting the intracellular PGE<sub>2</sub> pool prior to mechanical loading. Pharmacologic inhibition of both Cox1 and Cox2 via indomethacin treatment, or selective inhibition of Cox2 alone via NS-398 treatment, was found to reduce osteogenic response to loading conducted several hours after administration of the inhibitors [162]. This result has been confirmed *in vitro* using fluid shear and stretch, where PGE<sub>2</sub> levels can more easily be measured from cell culture media [163–165]. The mechanism of PGE<sub>2</sub> release from mechanically stimulated cells is controversial, and might involve the opening of large, pore-forming connexin-43 hemichannels [166] or the purinergic P<sub>2</sub>X<sub>7</sub> protein complex [167, 168]. Once released, PGE<sub>2</sub> binds in an autocrine or paracrine fashion to the heptahelical Ep receptors (Ep1-4), which mediate its effects [169, 170]. In summary, both *in vivo* and *in vitro* experiments point to a prominent role for prostaglandins in bone cell mechanotransduction, but the mechanism by which PGE<sub>2</sub> is released, and which receptors are important for its paracrine/autocrine effects, are unclear.

**Wnt signaling**—More recently, the Wnt (Wingless-related integration site) signaling pathway has been identified as a major intermediate player in bone cell mechanotransduction [171]. The Wnt family of secreted glycoproteins is made up of 19 different Wnt genes (in humans) whose protein products can activate multiple signaling pathways (reviewed in [172]). Canonical Wnt signaling involves secreted Wnts activating a receptor complex comprising a single-pass low-density lipoprotein receptor-related protein (Lrp) and a heptahelical Frizzled (Fzd) receptor which subsequently leads to the activation of the Dishevelled (Dsh) phosphoprotein [173]. Activated Dsh inhibits GSK3β activity which, in turn, results in reduced GSK3β-mediated phosphorylation of the transcription factor β-catenin and thus reduced β-catenin degradation [173]. This allows the stabilized β-catenin to act as a transcriptional co-activator by forming complexes with members of the

TCF/LEF family of transcription factors and increasing the expression of various Wnt/ $\beta$ -catenin target genes [173]. Importantly, numerous target genes of  $\beta$ -catenin are associated with enhanced osteogenesis and reduced resorption. Using a reporter construct, mechanical loading was shown to activate  $\beta$ -catenin mediated transcription both *in vivo* and *in vitro* [174, 175]. Remarkably, osteocytes appear to be the first cells to exhibit activated  $\beta$ -catenin transcriptional activity after loading [176], suggesting that Wnt signaling in osteocytes might be a sensor cell response pathway. Moreover, deletion of one of the canonical Wnt co-receptors-Lrp5-in mice appears to prevent load-induced bone formation [177, 178], which suggests that Lrp5/ $\beta$ -catenin signaling is vitally important for mechanotransduction in bone. The canonical Wnt pathway is negatively regulated by a number of endogenous secreted inhibitors that bind Lrp5/6 or Wnts. Among these inhibitors is a potent Lrp5/6 antagonist-sclerostin-that is highly expressed by mature osteocytes but not by other bone cells (e.g., osteoblasts, osteoclasts, bone lining cells). Sclerostin, the protein product of the *SOST* gene, is significantly down-regulated by mechanical loading, and significantly up-regulated by mechanical disuse [179]. Forced overexpression of a *SOST* transgene prevents load-induced bone formation [180], whereas *Sost* deletion improves mechanotransduction [181]. Moreover, inhibition of *Sost* during mechanical disuse prevents bone loss [182]. In summary, Wnt has been identified as a major mediator of bone cell mechanotransduction. The modulation of numerous secreted Wnt inhibitors, as well as the downstream signaling nodes activated by Wnt, are still being defined.

**IGF-1 signaling**—Unlike skeletal muscle, in which IGF-1 does not appear to be necessary for the mechanically-induced increase in mTORC1 signaling and skeletal muscle mass, bone cell mechanotransduction requires IGF-1 signaling for the anabolic effects of loading. For instance, IGF-1 is known to play an essential role in embryonic bone development [183, 184] and early studies also suggested a potential role for IGF-1 in the regulation of bone mass in response to changes in mechanical loading. Specifically, studies conducted in mechanically stimulated osteocytes *in vitro*, and in mechanically stimulated rat vertebrae and tibia *in vivo*, revealed increased expression of IGF-1 mRNA expression after a bout of increased loading [185–187]. Furthermore, it was shown IGF-1 administration increases osteoblast proliferation *in vivo* [188] and that transgenic overexpression of IGF-1 in osteoblasts resulted in enhanced responsiveness to *in vivo* mechanical loading in mice [189]. Moreover, conditional disruption of the IGF-1 gene in type 1 $\alpha$ (2) collagen-expressing cells *in vivo* effectively eliminated the osteogenic responses to increased mechanical loading in mice [190]. More recently, conditional osteocyte knockout of IGF-1 also ablated the osteogenic response to mechanical loading and this occurred despite normal IGF-1 expression in osteoblasts [191]. In reloading models, recombinant IGF-1 infusion has been shown to be sufficient to enhance the differentiation of osteoblast precursor cells and increase trabecular bone formation in rats subjected to mechanical unloading [192], while osteocyte IGF-1 receptor expression was found to be necessary for reloading-induced periosteal bone formation in mice [193]. Combined, these data suggest that IGF-1 does indeed play a critical role in the mechanical regulation of bone mass. However, the downstream effectors of activated IGF-1 are less clear. For instance, *in vitro* IGF-1 treatment stimulates the activation of the IGF-1 receptor, Ras, ERK1/2 and Akt in cultured BMOp cells isolated from normally loaded bones [188], but the downstream targets of these

signaling events is unknown. In another study, conditional knockout of osteocyte IGF-1 reduced the load-induced increase in Wnt signaling-related gene expression and  $\beta$ -catenin protein levels. It also increased the loading-induced expression of the SOST gene and inhibited the load-induced increase in Cox-2 expression [191]. In summary, the IGF-1 axis is clearly important for mechanical signaling in bone cells, with more limited evidence suggesting that IGF-1 may regulate Wnt and PG signaling in response to increased mechanical loading. More studies are needed, however, to further investigate other possible IGF-1 mediated signaling pathways (e.g. Ras/ERK and Akt signaling).

**Nitric oxide signaling**—Similar to skeletal muscle, another pathway activated by mechanical stimulation in bone is nitric oxide signaling [194]. Nitric oxide (NO) is a free radical and as such can diffuse through the plasma membrane freely. NO is generated from the amino acid L-arginine by one of three isoforms of Nitric Oxide Synthase (NOS). *In vitro*, NO is released from mechanically stimulated osteoblasts and osteocytes [195–197]. *In vivo*, depletion of the NO pool prior to mechanical loading in rats using one of several NOS inhibitors significantly impairs the osteogenic response to loading [198]. Moreover, in mice lacking the inducible form of NOS (iNOS<sup>-/-</sup>), a return to normal cage floor locomotion following 7 days of tail suspension failed to generate an otherwise osteogenic response, as was seen in iNOS replete mice [199]. These experiments suggest that the osteoblastic response to mechanical stimulation requires NO signaling. Perhaps equally noteworthy is the effect that NO signaling has on bone resorption. In addition to enhancing bone formation, mechanical loading impairs bone resorption and NO signaling might be one of the main mechanisms by which it is accomplished. For instance, mechanically-induced increases in NO cause a decrease in RankL which is a major regulator of osteoclast development and survival [200]. In summary, while NO appears to be an important second messenger in bone cell mechanotransduction, its target enzymes and cell type of origin (i.e., stromal cells, osteocytes, lining cells) are still open to investigation.

In conclusion, numerous second messenger cascades are activated in bone tissue during and after mechanical stimulation, including IGF-1, prostaglandins, nitric oxide, and components of the Wnt pathway. How these various cascades interact with one another is unclear. Recent experimental work has begun to link some of these otherwise disparate pathways together. For example, Kitase et al [201] have shown that mechanically induced PGE<sub>2</sub> in osteocytes participates in an autocrine loop that ultimately inactivated GSK3 $\beta$  and stimulates the downstream canonical Wnt target  $\beta$ -catenin. It remains to be determined whether other major cascades in bone cell mechanotransduction are linked, or whether they act as accessory pathways in the event that one is impaired. The need for clear delineation of the processes involved is obvious: recapitulation of the mechanotransduction cascades using non-mechanical stimuli holds great promise in preventing fractures, particularly in those with frail skeletons that could not endure significant loading episodes.



## SIMILARITIES AND DIFFERENCES BETWEEN SKELETAL MUSCLE AND BONE

While the differences in mechanotransduction mechanisms between skeletal muscle and bone appear to be great, there is also evidence that some common mechanisms might exist. For example, recent studies suggest that mechanically sensitive ion channels, such as TrpP1 channels in bone and TrpV1 channels in skeletal muscle, may play significant roles in the anabolic response to increased mechanical loading. Moreover, a role for these mechanically sensitive ion channels suggests that ions, such as  $\text{Ca}^{2+}$ , may be an important second messenger in both of these tissues. In addition, NO, albeit from potentially different sources (iNOS in bone and nNOS in skeletal muscle), also appears to be involved in load-induced increases in bone and skeletal muscle mass. However, there are also mechanically sensitive mechanisms that are established in one tissue but remain to be convincingly demonstrated in the other tissue (see Table 1). For example, as described above, it has been convincingly shown that mTORC1 signaling plays a fundamental role in mechanically-induced increases in skeletal muscle mass; however, its role in bone mechanotransduction remains to be determined. Furthermore, there is compelling evidence that PG and Wnt signaling are mechanically sensitive in bone and that they play important roles in load-induced increases in bone mass; but whether these molecules play a role in the mechanical regulation of skeletal muscle mass remains unclear. Therefore, in the hope of stimulating new research into these questions we briefly review the evidence for a potential role for PG and/or Wnt signaling in the mechanical regulation of skeletal muscle mass, and for a potential role of mTORC1 signaling in the mechanical regulation of bone mass.

### A Role for Prostaglandins in the Mechanical Regulation of Skeletal Muscle Mass?

PGs are known to be produced in skeletal muscle and incubation with AA or  $\text{PGF}_{2\alpha}$  has been shown to stimulate an increase in protein synthesis in isolated skeletal muscles and to induce hypertrophy of cultured myotubes [202–207]. Moreover, the  $\text{PGF}_{2\alpha}$ -induced myotube hypertrophy was recently shown to be mediated through a PI3K/ERK/mTORC1-dependent mechanism [204]. Importantly, numerous studies have shown that mechanical stimulation of skeletal muscle induces an increase in PGs, including  $\text{PGF}_{2\alpha}$  (for reviews see [208, 209]). Thus, an increase in PG synthesis and secretion could play a role in the mechanical activation of mTORC1, protein synthesis and muscle hypertrophy. Indeed, high doses of the general COX inhibitors, acetaminophen and ibuprofen, have been shown to be sufficient to inhibit the increase in protein synthesis that occurs following eccentric exercise in humans [210]. In contrast, ‘over-the-counter’ doses of general COX inhibitors do not inhibit resistance training-induced increases in muscle mass [208]. Furthermore, a COX-2 isoform specific inhibitor (NS398) has been shown to blunt synergist ablation-induced muscle hypertrophy in mice without inhibiting the activation of mTORC1 signaling [211]. Finally, not all PGs stimulate protein synthesis. For example,  $\text{PGE}_2$  (and AA) stimulates protein degradation in skeletal muscle [203]. These disparate findings highlight the need for more studies, using a range of experimental models, to clarify the role of different PGs in the mechanical regulation of protein turnover and skeletal muscle mass. Moreover, given the current evidence that PI3K and ERK signaling are not required for the mechanical activation of mTORC1 signaling, this would suggest that the role of PGs in the

mechanical regulation of skeletal muscle mass may be exerted through an mTORC1-independent mechanism.

### **A Role for Wnt Signaling in the Mechanical Regulation of Skeletal Muscle Mass?**

Wnt molecules play critical roles in the embryonic development of skeletal muscle and in the regulation skeletal muscle regeneration [212, 213]. Importantly, acute maximal exercise has been shown to increase Dsh/GSK3 $\beta$  interaction, and decrease GSK3 $\beta$  activity and  $\beta$ -catenin phosphorylation in humans [214]. Furthermore, resistance training-induced muscle fiber hypertrophy is associated with an increased expression of various Wnt signaling pathway members (e.g. Wnt1,  $\beta$ -catenin, LEF1, Cyclin D1) [215]. In mice, chronic mechanical overload-induced muscle hypertrophy is associated with an increase in  $\beta$ -catenin, Fzd-1, DSH-1 and LEF-1 protein expression, increased nuclear abundance of  $\beta$ -catenin and LEF-1, and increased expression of Wnt/ $\beta$ -catenin target genes c-myc and cyclin D1 [216]. Moreover,  $\beta$ -catenin has been shown to be necessary for chronic overload-induced muscle fiber hypertrophy [217]. Thus, current evidence strongly suggests that canonical Wnt/ $\beta$ -catenin signaling plays a significant role in mechanically-induced skeletal muscle growth. More recently, evidence has also demonstrated a link between Wnt signaling, muscle hypertrophy and the activation of mTORC1 signaling [218, 219]. Specifically, the overexpression of Wnt7a (but not Wnt 3a or 5a) induced hypertrophy of muscle fibers *in vivo*, and in myotubes that was independent of myoblast cell proliferation, differentiation and fusion [218]. This Wnt7a-induced hypertrophy was found to be associated with a non-canonical Wnt activated signaling pathway that required Fzd7 and G protein subunit  $\alpha_s$ , and involved the activation of PI3K/Akt/mTORC1 signaling, independent of the IGF-1 receptor or IRS1 activation [218]. Moreover, Wnt7a-induced myotube hypertrophy was inhibited by rapamycin [218]. Overall, there is significant data implicating canonical Wnt signaling in the mechanical regulation of skeletal muscle mass, while more recent data suggests a possible role for non-canonical Wnt signaling in the activation of mTORC1 signaling. However, while it is tempting to speculate that the non-canonical Wnt signaling-induced activation of mTORC1 could potentially play a role in the mechanical activation of protein synthesis and skeletal muscle hypertrophy, several important questions remain to be answered. For example, are Wnt7a expression and/or secretion increased during or after different types of acute and chronic mechanical stimulation? Given the current evidence that the acute mechanical activation of mTORC1 occurs in a PI3K/Akt-independent manner, perhaps a Wnt7a could contribute to the more delayed (hours) activation of mTORC1. If so, what is the cellular origin of the secreted Wnt7a: skeletal muscle cells, cells of the extra cellular matrix and/or immune cells? Thus, while promising, significantly more work is required to establish a role, if any, for non-canonical Wnt signaling in the mechanical activation of mTORC1, protein synthesis and skeletal muscle growth.

### **A Role for mTORC1 in the Mechanical Regulation of Bone Mass?**

Given the predominant role that mTORC1 signaling plays in mechanically-induced skeletal muscle growth, it is of interest to examine whether there is any evidence for a similar role for mTORC1 in the mechanical regulation of bone mass. To date, there is some evidence that mTORC1 does indeed play a role in bone growth and, more specifically, in the regulation of long bone length. For example, several studies have shown that the mTORC1

inhibitor, rapamycin, inhibits long bone growth in young rodents, possibly by directly inhibiting chondrocyte differentiation and/or indirectly by inhibiting growth plate angiogenesis which leads to reduced chondrogenesis [220–224]. Thus, mTORC1 may play a vital role in long bone growth providing a mechanism for mitogens and nutrients to stimulate bone growth via increased growth plate activity. Importantly, recent evidence also implicates mTORC1 in mechanically-induced cartilage growth [225]. Specifically, the mechanical activation of mTORC1 was found to be necessary for cell proliferation, chondrogenesis, and cartilage growth during embryonic bone development [225]. Other evidence suggests that mTORC1 not only regulates chondrocytes, but also regulates osteoblasts. For instance, rapamycin has been shown to inhibit the proliferation and differentiation of preosteoblastic cells, in part, by inhibiting the expression of cyclins A and D1, and the transcription factor Runx2, respectively [226]. Furthermore, rapamycin has also been reported to inhibit erythropoietin-induced osteoblast differentiation in some preosteoblastic cell lines [227]. These data suggest that mTORC1 could play an important role on regulating bone mass, in part, by modulating the abundance of osteoblasts. This is further supported by a recent study that implicated mTORC1 in a Wnt signaling pathway that enhances post-natal bone mass via an increase in osteoblast numbers and activity [228]. Specifically, in this study, the induction of Wnt7b expression, either during embryonic development or at 4 wk post-natal, resulted in profound increases in bone mass that were related to increased osteoblast numbers [228]. Furthermore, it was found that Wnt7b (and Wnt3a) activated mTORC1 signaling, *in vitro* and *in vivo*, via a non-canonical PI3K/Akt pathway, and that the inducible deletion of the mTORC1 component, Raptor, markedly reduced the Wnt7b-induced increase in osteoblast activity and bone mass [228]. Interestingly, rapamycin analogs (e.g., evirolimus) appear to inhibit osteoclast survival and activity, suggesting that the activation of mTORC1 might also increase bone resorption [229]; however, the Wnt7b-induced increase in mTORC1 signaling and bone mass was not associated with changes in bone resorption [228]. These exciting data suggest that the activation of mTORC1 may indeed play a role in the regulation of bone mass by stimulating an increase in osteoblast numbers and activity.

In summary, an expanding body of evidence suggests that mTORC1 plays an important role in promoting long bone growth by increasing chondrocyte proliferation and growth plate activity. Importantly, mTORC1 has also been shown to promote increases in developmental and post-natal bone mass by stimulating an increase in osteoblast proliferation, differentiation and activity; however, there is currently no direct evidence linking mTORC1 signaling with mechanically-induced increases in osteoblast function and bone mass. Nevertheless, given the evidence that Wnt signaling plays a major role in mechanotransduction in bone, and the recent finding that Wnt7a is sufficient to increase bone mass via an mTORC1-dependent mechanism, future research may lead to the establishment of mTORC1 as a *bona fide* regulator of mechanically-induced increases in bone mass.

## CONCLUSION

In conclusion, the molecular mechanisms that regulate mechanotransduction in skeletal muscle and bone are complex, with multiple mechanisms likely operating in synergy. In

skeletal muscle mTORC1 has been established as a central mediator of mechanically-induced changes in protein synthesis and muscle cell size, with several putative candidates proposed to play a role in mTORC1's activation (e.g. PA, amino acids, TSC2 translocation,  $\text{Ca}^{2+}$  and NO). However, despite these advances, still very little is known about the upstream mechanically-activated sensor(s) that ultimately trigger these second messengers to promote mTORC1 signaling. In bone, a much more complicated picture of mechanotransduction has emerged with the necessity to consider the role of multiple cell types in this process. Nonetheless, current evidence has shown that mechanically-sensitive ion channels and G-protein coupled receptors play significant roles in detecting changes in mechanical stress in bone mass. Furthermore, IGF-1, NO, PG and Wnt signaling have all been implicated as second messengers in bone mechanotransduction. Major questions that remain to be resolved include whether PG and non-canonical Wnt signaling plays a role in the mechanical activation of mTORC1 in skeletal muscle and whether mTORC1 signaling is involved in bone mechanotransduction. This review highlights that there is still much to be discovered about the process of mechanotransduction in skeletal muscle and bone. By examining the differences and similarities in mechanotransduction between these two tissues it is hoped that this review will stimulate new insights and ideas for future research and collaboration between bone and muscle biologists. Ultimately, advances in our knowledge of the mechanisms of mechanotransduction will assist in the development of effective exercise and pharmaceutical strategies aimed at increasing, and/or preventing the loss of, bone and skeletal muscle mass.

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## ABBREVIATIONS

<b>AA</b>	arachidonic acid
<b>Akt</b>	v-Akt Murine Thymoma Viral Oncogene
<b>BAPTA-AM</b>	1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid tetrakis(acetoxymethyl ester)
<b>CaMKK<math>\alpha</math></b>	$\text{Ca}^{2+}$ /calmodulin-dependent protein kinase alpha
<b>Cox</b>	cyclooxygenase
<b>DGK</b>	diacylglycerol kinase
<b>DGK<math>\zeta</math></b>	diacylglycerol kinase zeta
<b>Dsh</b>	Dishevelled
<b>ERK</b>	extracellular signal-regulated kinase
<b>FRB</b>	FKBP12-rapamycin binding
<b>FRET</b>	fluorescence resonance energy transfer
<b>Fzd</b>	Frizzled

<b>GAP</b>	GTPase activating protein
<b>Gd<sup>3+</sup></b>	gadolinium
<b>GDP</b>	guanosine diphosphate
<b>GPCR</b>	G-protein coupled receptor
<b>GSK3<math>\beta</math></b>	glycogen synthase kinase 3 beta
<b>GTP</b>	Guanosine-5'-triphosphate
<b>H<sub>2</sub>O<sub>2</sub></b>	hydrogen peroxide
<b>IGF-1</b>	insulin-like growth factor 1
<b>iNOS</b>	inducible nitric oxide synthase
<b>LEF</b>	Lymphoid enhancer-binding factor
<b>LEL</b>	late endosomal/lysosomal
<b>L-NAME</b>	L-N <sup>G</sup> -Nitroarginine methyl ester
<b>LPAAT<math>\theta</math></b>	lysophosphatidic acid acyltransferase theta
<b>Lrp</b>	lipoprotein receptor-related protein
<b>MAPK</b>	mitogen-activated protein kinase
<b>MEK</b>	Mitogen/Extracellular signal-regulated Kinase
<b>MGF</b>	mechano growth factor
<b>mTOR</b>	mechanistic or mammalian target of rapamycin
<b>mTORC1</b>	mTOR complex1
<b>mTORC2</b>	mTOR complex2
<b>NO</b>	nitric oxide
<b>NOS</b>	nitric oxide synthase
<b>nNOS</b>	neuronal nitric oxide synthase
<b>Nox4</b>	NADPH oxidase 4
<b>O<sub>2</sub><sup>-</sup></b>	superoxide
<b>ONOO<sup>-</sup></b>	peroxynitrite
<b>p90RSK</b>	90 kDa ribosomal S6 kinase
<b>PA, PDL1</b>	phospholipase D1; phosphatidic acid
<b>PG</b>	prostaglandin
<b>PI3K</b>	phosphatidylinositide 3-kinase
<b>Pkd</b>	polycystic kidney disease
<b>PTH1R</b>	parathyroid hormone 1 receptor

<b>RAF</b>	rapidly accelerated fibrosarcoma
<b>RANKL</b>	Receptor activator of nuclear factor kappa-B ligand
<b>RAS</b>	rat sarcoma oncogene
<b>Rheb</b>	Ras homologue enriched in the brain
<b>RNS</b>	reactive nitrogen species
<b>ROS</b>	reactive oxygen species
<b>RUNX2</b>	Runt-related transcription factor 2
<b>SR</b>	sarcoplasmic reticulum
<b>SC</b>	satellite cells
<b>TCF</b>	T-cell factor
<b>Trp</b>	transient receptor potential
<b>TrpV1</b>	transient receptor potential cation channel subfamily V1
<b>TrpV4</b>	transient receptor potential cation channel subfamily V4
<b>TSC2</b>	Tuberous Sclerosis Complex 2
<b>Vps34</b>	vacuolar protein sorting 34
<b>Wnt, Wnt</b>	Wingless-related integration site.

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### Highlights

- We review the primary molecular mechanisms that have been implicated in skeletal muscle and bone mechanotransduction.
- mTORC1 signaling plays a fundamental role in the mechanical regulation of skeletal muscle mass and has several putative activators.
- GPCRs, ion channels, prostaglandins, IGF-1, Wnts and NO play roles in bone mechanotransduction.
- It remains to be determined whether mTORC1 signaling plays a role in the mechanical regulation of bone mass.
- It remains unknown whether prostaglandins and/or Wnts play a role in the mechanical activation of mTORC1 in skeletal muscle.

**Table 1**Summary of the pathways/molecules involved in muscle and/or bone mechanotransduction<sup>1,2</sup>

	Muscle	Bone
<b>mTORC1</b>	✓✓✓	
<b>IGF-1</b>	✓	✓✓
<b>Erk1/2</b>	✓	✓✓
<b>PA</b>	✓✓	
<b>TSC2/Rheb</b>	✓	
<b>iCa<sup>2+</sup></b>	✓	✓✓✓
<b>ROS/RNS</b>	✓	✓✓
<b>AA</b>	✓	
<b>GPCRs</b>		✓
<b>PGs</b>	✓	✓✓
<b>Wnt</b>	✓	✓✓✓

<sup>1</sup> the number of check marks indicate the strength of the role in mechanotransduction (based on the literature).<sup>2</sup> see text for full names of abbreviated molecules